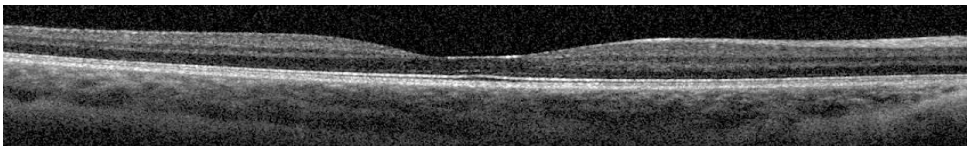
UNIVERSITAT POLITÈCNICA DE CATALUNYA
BARCELONATECH
Facultat d'Òptica i Optometria de Terrassa

MÀSTER UNIVERSITARI EN OPTOMETRIA I CIÈNCIES DE LA VISIÓ

TREBALL FINAL DE MÀSTER

ESTUDI DEL GRUIX DE LA ONL EN PACIENTS AMB DRUSES RETICULARS I TOVES AL LLARG DEL TEMPS



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Terrassa, a 19 de Juny de 2017

MÀSTER UNIVERSITARI EN OPTOMETRIA I CIÈNCIES DE LA VISIÓ

El Sr. Genís Cardona Torradeflot, com a director del treball

CERTIFICA,

Que la Sra. **Clara Ramon Clar** ha realitzat sota la seva supervisió el treball ESTUDI DEL GRUIX DE LA ONL EN PACIENTS AMB DRUSES RETICULARS I TOVES AL LLARG DEL TEMPS, que es recull en aquesta memòria per optar al títol de Màster en Optometria i Ciències de la Visió.

I per a què consti, signo aquest certificat.

Sr Genís Cardona Torradeflot
Director del treball

Terrassa, 5 de juny de 2017

UNIVERSITAT POLITÈCNICA DE CATALUNYA
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MÀSTER UNIVERSITARI EN OPTOMETRIA I CIÈNCIES DE LA VISIÓ

ESTUDI DEL GRUIX DE LA ONL EN PACIENTS AMB DRUSES RETICULARS I TOVES AL LLARG DEL TEMPS

RESUM

PROPÒSIT: Avaluar els canvis que apareixen en el gruix de la Capa Nuclear Externa (ONL) de la retina en dos grups diferenciats de pacients: pacients amb druses toves i pacients amb druses reticulars, en un període de 2 anys.

MÈTODES: Es va mesurar el gruix de la ONL a pacients >50 anys prèviament diagnosticats de druses toves (n=17) o de druses reticulars (n=9). La mesura es va realitzar a partir de volums de tomografia ocular computeritzada (SD-OCT, Spectralis, Heidelberg Engineering), on el propi programa defineix el gruix de les diferents capes de la retina. En la majoria de casos, quan la mida de les druses impedia la mesura automàtica, es va haver de redibuixar manualment els límits de la ONL. Es van agafar mesures de 3 visites diferents, la primera d'una visita inicial (V0), una al cap d'un any (V1) i la darrera al cap de dos anys (V2).

RESULTATS: El gruix promig de la ONL a la V0 en pacients amb druses toves va ser de 65.3±7.1 µm i de 57.6±7.3 µm en pacients amb druses reticulars (p=0.016), a la V1 de 64.7±7.6 µm al grup de druses toves i de 55.7±7.5 µm al grup de druses reticulars (p=0.009), i a la V2 de 66.2±12.9 µm per pacients amb druses toves i de 53.4±9.7 µm per pacients amb druses reticulars (p=0.009). En totes les visites, la major diferència entre els dos grups es va trobar en els sectors més perifèrics (p<0.05). Als 2 anys, l'aprimament més marcat es va observar en el sector central i superior de l'anell 1 en les druses toves (p=0.027 i p=0.032, respectivament), i en els sectors nasal i superior dels dos anells en pacients amb druses reticulars (p<0.05).

CONCLUSIONS: En aquest estudi, el gruix de la ONL s'ha vist reduït en presència de druses amb el pas del temps. En el grup de druses reticulars s'ha trobat un gruix de la ONL inferior al grup de les druses toves, fet que podria explicar el major impacte funcional que experimenten els pacients que tenen aquest tipus de druses en comparació als altres tipus.

MÀSTER UNIVERSITARI EN OPTOMETRIA I CIÈNCIES DE LA VISIÓ

ESTUDIO LONGITUDINAL DEL GROSOR DE LA ONL EN PACIENTES CON DRUSAS RETICULARES Y BLANDAS

RESUMEN

PROPÓSITO: Evaluar los cambios que aparecen en el grosor de la Capa Nuclear Externa (ONL) de la retina en dos grupos diferenciados de pacientes: pacientes con drusas blandas y pacientes con drusas reticulares, en un período de 2 años.

MÉTODOS: Se midió el grosor de la ONL a pacientes >50 años previamente diagnosticados de drusas blandas (n=17) o de drusas reticulares (n=9). La medida se realizó a partir de volúmenes de tomografía ocular computerizada (SD-OCT, Spectralis, Heidelberg Engineering), siendo el propio programa el que mide automáticamente el grosor de las capas de la retina. En la mayoría de casos, en los que el tamaño de la drusa impedía la medida automática, se tuvo que redibujar manualmente los límites de la ONL. Se obtuvieron datos de 3 visitas diferentes, la primera de una visita inicial (V0), otra al cabo de un año (V1) y una tercera al cabo de dos años (V2).

RESULTADOS: El grosor de la ONL en la V0 en pacientes con drusas blandas fue de 65.3±7.1 y de 57.6±7.3 en pacientes con drusas reticulares (p=0.016), en la V1 de 64.7±7.6 en el grupo de drusas blandas y de 55.7±7.5 en el grupo de drusas reticulares (p=0.009), y en la V2 de 66.2±12.91 para pacientes con drusas blandas y de 53.4±9.7 para drusas reticulares (p=0.009). En todas las visitas, la mayor diferencia entre ambos grupos se encontró en los sectores más periféricos (p<0.05). A los 2 años, el mayor adelgazamiento se observó en el sector central y superior del anillo 1 en drusas blandas (p=0.027 i p=0.032, respectivamente), y en los sectores nasales y superiores de los dos anillos en drusas reticulares (p<0.05).

CONCLUSIONES: En este estudio, el grosor de la ONL se encontró reducido en presencia de drusas con el paso del tiempo. El grupo de drusas reticulares mostró tener un grosor inferior al grupo de drusas blandas, hecho que podría explicar el mayor impacto funcional que han mostrado tener este tipo de pacientes en comparación con otros tipos de drusas.

MÀSTER UNIVERSITARI EN OPTOMETRIA I CIÈNCIES DE LA VISIÓ

LONGITUDINAL STUDY OF THE THICKNESS OF THE OUTER NUCLEAR LAYER IN PATIENTS WITH SOFT AND RETICULAR DRUSEN

ABSTRACT

PURPOSE: The main purpose of this study was to compare the longitudinal changes in the thickness of the retina Outer Nuclear Layer (ONL) in two different groups of patients, one group with soft drusen and the other group with reticular pseudodrusen, in a period of 2 years.

METHODS: the thickness of the ONL of patients >50 years previously diagnosed with soft drusen (n=17) or reticular pseudodrusen (n=9) was measured. Measurements were obtained from scan volume from ocular coherence tomography images (SD-OCT, Spectralis, Heidelberg Engineering), in which the software presents automatic measures of the thickness of all retina layers. In those cases in which the size of the drusen impeded automatic measurement, it was necessary to manually redraw the limits of ONL Layer. Three different measures were obtained, one from a basal visit (V0), another from year one (V1) and the third one was at two years after diagnosis (V2).

RESULTS: ONL thickness in V0 in patients with soft drusen was 65.3±7.1µm and 57.6±7.3 µm in reticluar pseudodrusen patients (p=0.016); in V1, thickness was 64.7±7.6 µm in soft drusen and 55.7±7.5 µm in reticluar pseudodrusen (p=0.009); and in V2, it was 66.2±12.91 µm in patients with soft drusen and 53.4±9.7 µm in patients with reticular pseudodrusen (p=0.009). In all visits, the main difference between both groups was in the peripheral sectors (p<0.05). After 2 years, the main thinning in the soft drusen group was in the central sector and superior sector of ring 1 (p=0.027 and p=0.032, respectively), and in the nasal and superior sectors of ring 1 and 2 in the reticular pseudodrusen group (p<0.05).

CONCLUSIONS: In the present study, ONL thickness was reduced in presence of drusen after 2 years of follow-up. ONL thinning was higher in the reticular pseudodrusen group, as compared with soft drusen. This finding may explain the major functional impact that patients with this type of drusen have, compared with other type of drusen.

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Finally, thank you to my family, mi Friends, and to Youssef.

Title: LONGITUDINAL STUDY OF THE THICKNESS OF THE OUTER NUCLEAR LAYER IN PATIENTS WITH SOFT AND RETICULAR DRUSEN

(Article formatted according to the instructions for authors of the journal **Retina**: http://edmgr.ovid.com/retina/accounts/ifauth.htm)

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INTRODUCTION

Age-Related Macular Degeneration (AMD) is the leading cause of blindness in people over 50 years in developed countries, with a prevalence of 3.4% in Spain[1]. The pathogenesis of AMD is not entirely clear, but it is known that drusen are a hallmark in the early and intermediate stages of the disease[2]. However, an eye with only a few small, hard drusen is not considered to have AMD, as this is a part of normal aging[3].

Drusen are deposits of trapped extracellular material lying between the basement membrane of the retinal pigment epithelium (RPE) and the inner collagenous zone of Bruch's membrane. Drusen are observed during direct or indirect ophthalmoscopy examinations as pale and yellowish spot lesions on the ocular fundus, and there are several types of them. The functional impact of these deposits on subjects' vision in the early stages of the disease is a decrease in sensitivity in microperimetry, a difficulty in dark adaptation and blurred vision, among others. This study was focussed on two different types of drusen: soft Drusen (SD) and reticular pseudodrusen (RPD). Soft drusen are bright-round spots, with yellowish and ill-defined margins, located under the RPE (between the basal membrane of the RPE and the inner layer of the Bruch's Membrane). Their distribution is random, but they are most commonly found at the macula. Softening of the margins of hard drusen occurs when the base spreads out along Bruch's membrane[4]. Soft drusen are seen in Optical Coherence Tomography (OCT) as sub-RPE deposits (**Figure 1**).

Reticular Pseudodrusen (RPD), also known as Reticular Drusen or Subretinal Drusenoid Deposits, are yellowish white lesions with discrete, interlacing or confluent shape. They are located in the subretinal space, on the internal surface of the RPE, where they were first described in 1990 by Mimoun et al, authors that named them "pseudodrusen best seen with blue light"[5]. This type of drusen is better visualized using red-free light and they present an appearance of an interlacing yellowish pattern. Reticular pseudodrusen are differentiated from other drusen because they are above the RPE. They are seen in OCT as subretinal deposits (**Figure 1**). This type of drusen carry a much higher risk of developing end-stage AMD than other AMD lesions, such as soft indistinct drusen[6]. Different studies suggest that, in eyes with early or intermediate AMD, reticular pseudodrusen occur less often in the foveal area compared with more eccentric locations, and more frequently between the upper edge of the fovea and the superior temporal vessels. In contrast with soft drusen, RPD do not regress with calcification or pigment changes, but evolve through different stages. Macular sensitivity is different between patients with soft drusen and patients with RPD, with a possible explanation being the alteration of photoreceptor functions in the presence of RPD[7]. Some authors speculate that there is a correlation between the damage in rods and the presence of subretinal deposits, with cones being less vulnerable than rods to this sort of damage[8].

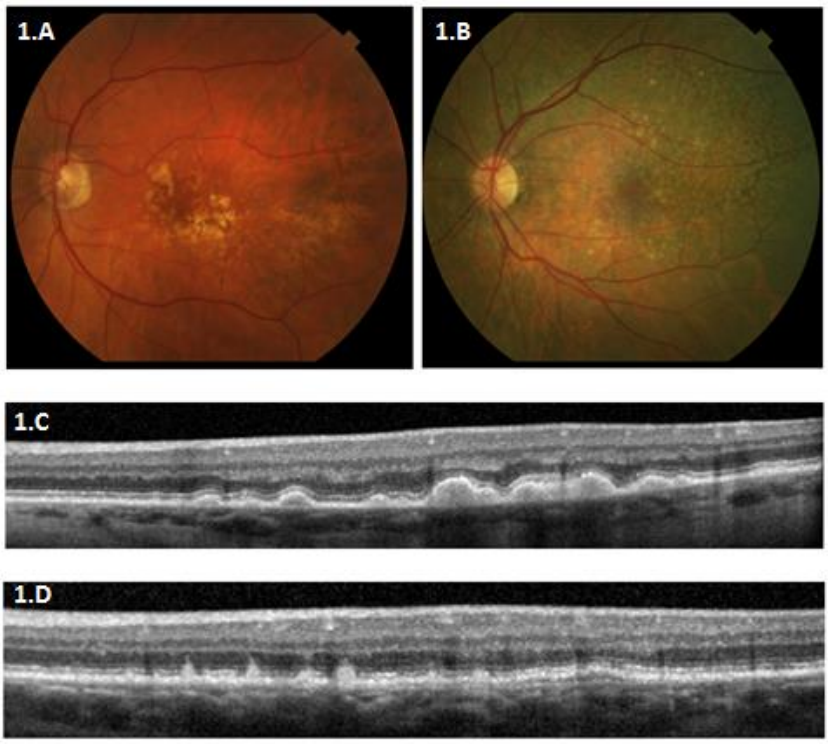

Figure 1. Examples of drusen signs in patients included in the study. 1.A and 1.B present ophthalmoscopic signs of soft drusen and reticular pseudodrusen in a retinography image. 1.C and 1.D show the corresponding OCT appearance of soft drusen and pseudodrusen, respectively

The Outer Nuclear Layer (ONL) is one of the 10 layers of the retina in which are located the nuclei of cone and rods photoreceptors. The area with the higher density of photoreceptors is the macula, providing a detailed vision of the images of external objects. The thickness of ONL is an important biomarker in retinal degenerations to evaluate their progression as direct mediators of vision. Previous studies have shown that the thickness of the ONL was significantly reduced in patients with drusen, above these deposits and other regions adjacent to the drusen. In effect, there seems to be a linear relationship between photoreceptor laminar decrease and drusen height[9].

Given the known functional impact of drusen, we aimed at assessing the structural causes of this impairment. The main purpose of this study was to compare the longitudinal changes in the ONL in two different groups of patients, one with soft drusen and one with reticular pseudodrusen, to determine possible differences between both types of drusen. Furthermore, the thickness of the ONL was examined in terms of topographic distribution to determine if some area is more susceptive to damage.

METHODS

Subjects of the study

Study design was observational and retrospective in nature. Patients were selected from those attending a busy ophthalmology centre (Institut de la Màcula, Barcelona, Spain), between May of 2009 and February of 2017. Patients were diagnosed by retina specialists and included in the study if they presented with either soft drusen or reticular pseudodrusen. To be included in the study patients had to have attended follow-up visits at 1 year and 2 years after initial diagnosis.

The present study was conducted according to the tenets Declaration of Helsinki. In addition, all patients included had signed the informed consent and data protection forms described in the Organic Law 15/1999 on Protection of Personal Data and the Law 21/2000 on the Rights of Information Concerning the Health and Autonomy of the Patient, which are given to all patients the first day they are visited in the centre.

Patients with spherical equivalent over ±6.00D were excluded from the study, as were those with retinal diseases which could affect ONL thickness (neovascular AMD, geographic atrophy, macular edema, epiretinal membrane, retinal dystrophies, etc.), patients previously treated with intravitreal injections, Laser photocoagulation or submitted to any type of intraocular surgery, with the exception of phacoemulsification with intraocular lens implantation surgery, if performed at least 3 months before the visit. Patients in treatment with medications that may have effects on the retina (Cloroquine, Hidroxicloroquine, Tamoxifen, Chlorpromazine, Thioridazine, Vigabatrin) and those in which bad quality Optical Coherence Tomography (OCT) images were obtained, such as patients with ocular opacities, were also excluded. However, it may be noted that exclusion and inclusion criteria were applied to eyes instead of patients, that is, in some instances one eye was included and the contralateral eye excluded.

Data was collected from 73 patients, 45 were patients with soft drusen and 28 were patients with reticular pseudodrusen. Considering the inclusion and exclusion criteria, however, only 26 patients were finally included in the study (17 with soft drusen and 9 with reticular pseudodrusen).

ONL thickness evaluation

Optical Coherence Tomography is a non-invasive imaging technique that delivers high-resolution images of the retina and sections of its layers. OCT was performed in both groups of patients with a Spectral-Domain OCT system (SD-OCT, Spectralis, Heidelberg Engineering). SD-OCT delivers significantly improved image resolution, in comparison to previous OCT image techniques and devices. Measurements of ONL thickness were conducted at each of the 9 map sectors defined by the Early Treatment Diabetic Retinopathy Study (ETDRS)[10] (**Figure 2**). Briefly, measurements were made at the centre of the macula and at two peripheral rings (ring 1 and ring 2). The size of the central ring is 1mm, the ring 1 is at 3mm and the ring 2 is at 6mm. At each ring, the four subfields (quadrants) were explored (superior, inferior, nasal, and temporal).

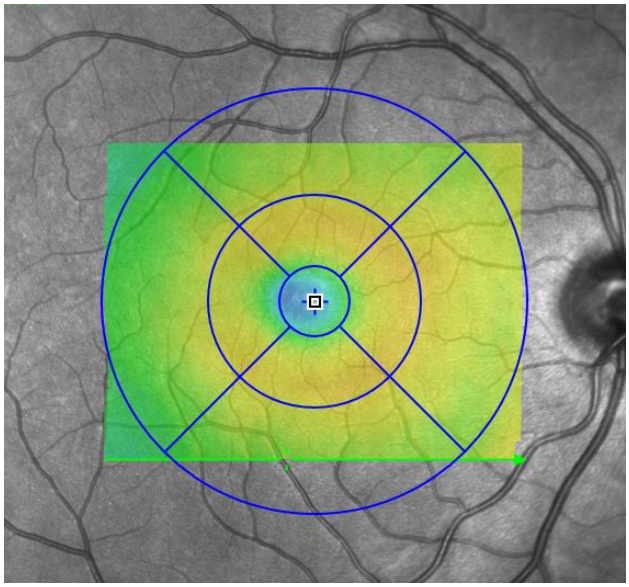

Figure 2. Example of the Spectralis SD-OCT ETDRS exploration grid shown in a healthy patient.

The OCT protocols used in this study considered either a volume of 19 or 37 sections. The choice of the protocol depended on the need for detail the retina specialist required on the occasion the patient was visited. Both protocols are satisfactory for the purposes of the present study. The Spectralis SD-OCT includes an on-board software (Heidelberg Eye Explorer, HEYEX Heidelberg Engineering) that performs automated segmentation of retinal boundaries to calculate retinal layers thickness, and provides an average value per quadrant or measurement location. However, in those cases in which the size of the drusen impeded automatic measurement, it was necessary to manually redraw the limits of ONL Layer. To proceed with manual measurements, the ONL was defined as the space between the Outer Plexiform Layer (OPL) and the Outer Limiting Membrane (OLM) (see **Figure 3**). To test reliability of manual measurements, 5 patients were selected at random and measurements were repeated by following the same procedure. When submitted to an Intraclass Correlation Analysis (ICC), results showed a high degree of consistency and agreement. Besides, the eye-tracking software in HEYEX minimises motion artefact during image acquisition and facilitates rescanning of the macula at the same retinal locus as baseline examination to ensure reliable follow-up measurements[11].

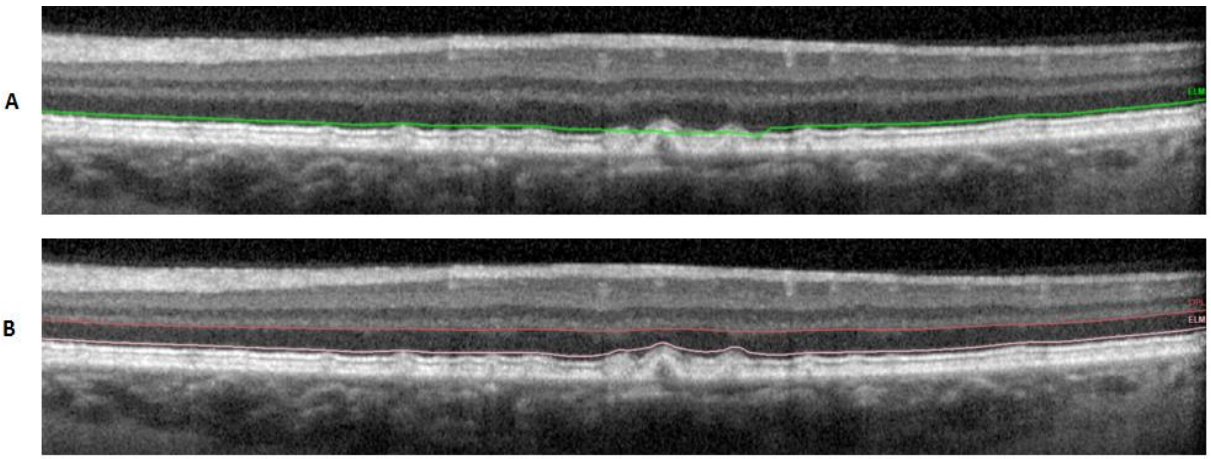

Figure 3. Image **A** shows the automatic OLM limit given by the Spectralis Program. Image **B** shows ONL thickness, as limited by OPL (superior line) and OLM (inferior line). The OLM was redrawn by the examiner in those cases in which drusen impeded real segmentation.

Three different measures were obtained corresponding to three different visits: the first one was at the time of diagnosis (Visit 0), the second corresponded to year one (Visit 1) and the third one was at two years after diagnosis (Visit 2). The time intervals elapsed between Visit 0 and Visit 1, and between Visit 1 and Visit 2, were of 12±2 months. All the OCT scans, as well as visual acuity data (measured with Topcon CC-100p), were obtained from the database of patients visited in the Institut de la Màcula (Barcelona). All measurements were conducted by the same experienced optometrist (C.R.C) and following exactly the same procedure.

Data analysis

Data analysis was conducted with the software IBM SPSS Statistics, version 22 (IBM Spain, S.A., Madrid). All data was explored for normality with the Kolmogorov-Smirnov test, revealing that most of the variables followed a normal distribution. Therefore, descriptive statistics was summarized as mean ± standard deviation (SD). In turn, inferential statistics was conducted with parametric tests.

Firstly, the non-matched pairs Students t-test was employed to explore the statistical significance of the differences found between both groups of patients (soft drusen and reticular pseudodrusen). This pair-wise comparison was conducted for each of the locations under study (central, nasal, temporal, superior and inferior of first ring and nasal, temporal, superior and inferior of second ring) and between the same visits (Visit 0, 1 and 2). Secondly, within each group of patients and visit, the ANOVA test was used to analyze the differences among the 9 different locations and, once a statistical significant difference was uncovered, a *post-hoc* test

of Bonferroni was employed for pair-wise analysis to determine the origin of the statistical differences. Finally, a matched-pairs Students t-test was used to explore differences between the three visits, that is, to determine the possible evolution of the condition. This analysis was conducted independently for each location and group of patients. A p-value of 0.05 was considered as the cut-off point for statistical significance.

RESULTS

A total of 26 eyes of 26 patients (7 right eyes, 19 left eyes) were included in the analysis, with 17 (3 male and 14 female) patients with soft drusen and 9 (1 male and 8 female) with reticular pseudodrusen. The mean age of the soft drusen group was 72.5±8.6 years (mean ± SD), with a range from 52 to 84 years, and 72.7±7.6 years in the reticular pseudodrusen group, with a range from 62 to 82 years. No statistically differences were found in age (p=0.955) and sex distribution (p=0.792) between both groups.

	Soft Drusen Group	**Reticular Pseudodrusen Group**	***P value***
Female (22)	14 (82.4%)	8 (88.9%)	0.955
Age	72.5±8.6 years	72.7±7.6 years	0.792

Table 1. Sex distribution and age of patients included in the study as classified in soft drusen and RPD groups.

Overall ONL thickness in V0 was 65.3±7.1µm in the soft drusen group and 57.6±7.3µm in RPD group (p=0.016); in V1 was 64.7±7.6µm in patients with soft drusen and 55.7±7.5µm in patients with RPD (p=0.009) and, in V2, 63.5±7.9µm in the soft drusen group and 53.4±9.7µm in the RPD group (p=0.009).

Best-corrected visual acuity (BCVA) was evaluated, and no statistically differences between the soft drusen and reticular pseudodrusen groups were found neither in V0 nor in V2 (p=0.29). Besides, within the same group, differences in VA between V0 and V2 were not statistically

significant. **Table 2** presents a summary of visual acuity measurements (in decimal notation) for each group of patients and visit.

	BCVA V0	**BCVA V2**	***P value***
Soft Drusen	0.95±0.14	0.91±0.19	0.31
Reticular Pseudodrusen	0.940±0.13	0.91±0.20	0.69

Table 2. Summary of BCVA in soft drusen and reticular pseudodrusen groups in Visit 0 and Visit 2.

Table 3 presents a summary of ONL thickness per location at Visit 0 for both groups of patients, together with the significance value (p-value) of the per-location differences between groups as analysed with a non-matched pairs Students t-test. Similar results are shown in **Table 4** and **Table 5** for Visit 1 and Visit 2. To facilitate the interpretation of the results, sectors were named N for nasal, S for superior, T for temporal and I for inferior, followed by the number 1 or 2, depending on the ring where measurements were conducted (**Figure 4**). The results of these tables are also shown in graphic format in **Figure 5**.

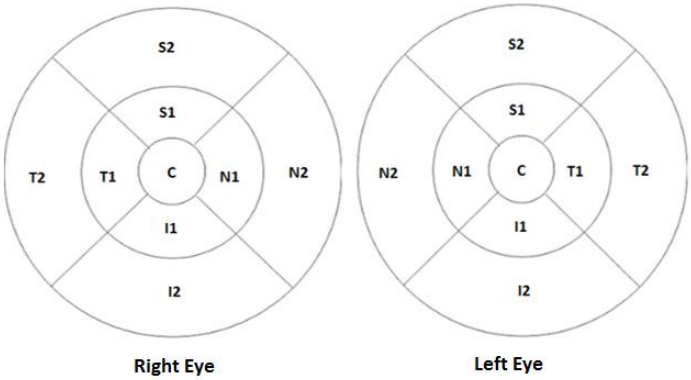

Figure 4. ETDRS Grid summary of the sectors examined in this study in the right eye and left eye.

	ONL thickness in soft drusen (µm)		ONL thickness in reticular pseudodrusen (µm)		
LOCATION	*Mean*	*SD*	*Mean*	*SD*	*p value*
Central	93.1	13.9	83.3	13.0	0.095
N1	70.3	10.1	62.8	14.9	0.138
S1	60.7	11.3	53.8	13.0	0.172
T1	67.5	8.5	62.9	10.9	0.245
I1	64.4	10.0	56.4	11.7	0.082
N2	56.5	7.3	47.9	8.6	**0.012**
S2	59.1	7.8	49.0	6.6	**0.004**
T2	58.4	5.5	52.2	5.8	**0.013**
I2	56.5	6.0	48.8	7.4	**0.012**

Table 3. Thickness of the outer nuclear layer (ONL) in soft drusen and reticular pseudodrusen at Visit 0. (N: nasal; T: temporal; I: inferior; S: superior; 1: ring 1; 2: ring 2). P-values in bold denote statistical significance.

	ONL thickness in soft drusen (µm)		ONL thickness in reticular pseudodrusen (µm)		
LOCATION	*Mean*	*SD*	*Mean*	*SD*	*p value*
Central	91.8	11.4	82.3	12.2	0.061
N1	70.0	11.2	58.4	15.6	**0.039**
S1	59.1	10.9	49.7	10.3	**0.042**
T1	66.5	7.7	63.0	11.7	0.363
I1	63.5	10.3	54.8	10.9	0.054
N2	56.2	8.6	45.7	10.0	**0.010**
S2	59.6	7.4	46.8	8.9	**0.001**
T2	57.9	6.1	52.2	7.3	**0.044**
I2	56.6	6.6	48.0	7.6	**0.009**

Table 4. Thickness of the outer nuclear layer (ONL) in soft drusen and reticular pseudodrusen at Visit 1. (N: nasal; T: temporal; I: inferior; S: superior; 1: ring 1; 2: ring 2). P-values in bold denote statistical significance.

	ONL thickness in soft drusen (µm)		ONL thickness in reticular pseudodrusen (µm)		
LOCATION	*Mean*	*SD*	*Mean*	*SD*	*p value*
Central	88.2	14.2	77.9	17.1	0.111
N1	68.1	11.9	54.7	19.9	**0.041**
S1	56.8	11.9	45.9	10.8	**0.030**
T1	67.8	8.8	61.3	11.6	0.125
I1	62.9	13.2	53.8	13.5	0.153
N2	55.9	8.9	44.6	12.1	**0.012**
S2	57.8	8.30	43.1	8.7	**<0.001**
T2	58.7	6.6	51.0	7.0	**0.011**
I2	55.8	7.7	48.0	9.0	**0.038**

Table 5. Thickness of the outer nuclear layer (ONL) in soft drusen and reticular pseudodrusen at Visit 2. (N: nasal; T: temporal; I: inferior; S: superior; 1: ring 1; 2: ring 2). P-values in bold denote statistical significance.

Mean values in **Tables 3**, **4** and **5** show that ONL thickness values were lower in all sectors in RPD patients with comparison with patients with soft drusen, although statistically significant differences ($p<0.05$) were only found in all sectors of ring 2 and all visits, at N1 and S1 in V1, and N1 and S1 at V2.

An ANOVA test was used to evaluate if there were differences among the nine sectors. This analysis was conducted visit by visit and for each group separately, revealing statistically significant differences in V0, V1 and V2 for the two groups of patients (all $p <0.001$).

In particular, for the soft drusen group and all visits, the most significant differences were found between the central position and all the other peripheral sectors ($p <0.001$), with thickness being higher in the central sector. In V0, there were differences between N1 and the four sectors of ring 2, with the ONL being thicker in the N1 sector; and between T1 and N2 ($p = 0.028$) and I2 ($p = 0.032$), with thick in T1. In V1, there were statistically significant differences between N1 and S1 ($p = 0.025$), being thicker S1, and between N1 and N2 ($p = 0.001$), T2 ($p = 0.007$) and I2 ($p = 0.002$), being thicker N1; as well as between T1 and N2 ($p = 0.044$), being thicker T1. With regards

to the reticular pseudodrusen group, statistically significant differences were also found between the central sector and almost all the other sectors in V0, V1 and V2, with the ONL being thicker in the central sector. In V1, a statistically significant difference in ONL thickness was found between T1 and N2 (p=0.040), which was not found in the other two visits, being thicker T1.

Comparing ONL thickness changes between visits for each sector and group separately, it was observed that changes were only statistically significant at the central and S1 sectors between V0 and V2 (p=0.027 and p=0.032, respectively) for patients with soft drusen, being thinner in V2. In the reticular pseudodrusen group differences were encountered at the N1 sector between V0 and V2 (p = 0.029), being thinner in V2; at the N2 sector between V0 and V1 (p = 0.010) and between V0 and V2 (p = 0.026), being thinner V2; at the S1 sector between V0 and V2 (p = 0.012), being thinner in V2; and at the S2 sector between V0 and V2 (p=0.001) and between V1 and V2 (p=0.003), being thinner V2.

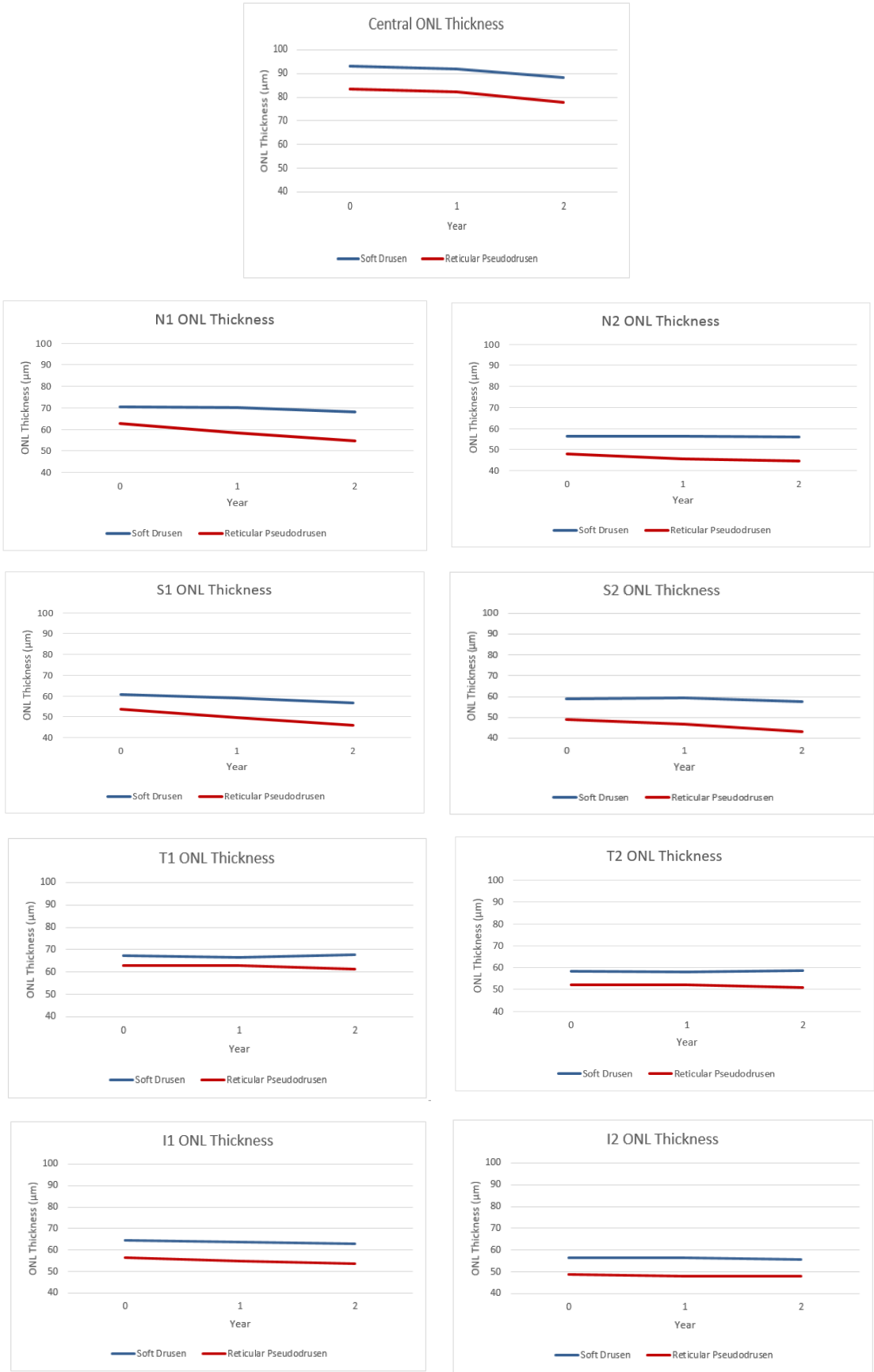

Figure 5. Representation of ONL thickness changes in each sector in Visit 0, Visit 1 and Visit 2.

DISCUSSION

Evaluation of general ONL thickness changes

The present results revealed that the average ONL thickness is lower in patients with reticular pseudodrusen than in those with soft drusen. A literature research failed to uncover previous studies investigating ONL thickness changes in these two groups of drusen. Schuman et al reported that drusen may affect photoreceptors by physical displacement, damaging the photoreceptor normal cell function and blocking the normal diffusion of metabolic components between photoreceptors and choroidal blood supply which is required to maintain the health of the outer retina and RPE[12]. Thus, the greater impact of pseudodrusen on ONL thickness as compared to soft drusen could have a mechanical origin given that, as reticular pseudodrusen are located between the RPE and the photoreceptor layer, they may act as a barrier for metabolic exchange between these structures, in contrast with soft drusen, which are located underneath the RPE. An alternative or complementary explanation could be related to a possible toxicity by direct contact of the actual pseudodrusen chemistry with the photoreceptors.

Evaluation of ONL thickness changes per sector in ETDRS Grid

Within the same visit, comparing sector-by-sector ONL thickness between both groups it was observed that the main differences were always at the peripheral sectors, in ring 2, as well as in the superior and nasal sectors at the one and two-year visit. Soft drusen are commonly located in the macular area, whereas reticular pseudodrusen are often seen in the superior macula. Considering the topographic distribution of drusen, it may be assumed that reticular pseudodrusen have a greater impact on ONL thinning than soft drusen. In a normal eye, photoreceptor distribution combines a high prevalence of cones in the fovea with a high prevalence of rods in the perimacular retina. With age, the density of photoreceptors is reduced in both areas. Previous studies speculated that cones would be less vulnerable as compared with rods to the occurrence of reticular pseudodrusen deposits, with a foveal involvement later in time[8-13]. Furthermore, some histological studies showed higher vulnerability of the rod system compared with a relative preservation of cone photoreceptors in the early stages of AMD in general[13].

In both groups of patients, in each visit, the central sector was statistically thicker in comparison to the other 8 sectors. Papay et al described that in healthy subjects the ONL is thicker in the macula and thickness decreases with eccentricity, where the numbers of cone cell bodies are decreasing but rod cell bodies are increasing[14]. For this reason, although central sector was thinned in the presence of soft drusen, this sector is significantly thicker compared to other sectors.

Although the present study didn't include a control group, a previous published study exposed ONL thickness in healthy subjects, where the foveal ONL thickness was 111.3±10.5 µm (mean±SD), S1 sector was 62.3±4.9 µm, I1 sector was 56.3±3.6 µm, T1 sector was 57.4±4.6 µm and N1 sector was 58.1±4.5µm[9]. With these results, it is confirmed that ONL is thicker in the foveal area and decreases with eccentricity. Besides, it may be observed that, in general, our patients had thinner ONL values than those reported in this study.

Evaluation of ONL thickness changes after 2 years of follow-up

When differences between the three different visits were evaluated in each sector and within each group of patients, it was observed that ONL central thickness in soft drusen group was statistically reduced after 2 years of follow up (p=0.027). In reticular pseudodrusen, thinning with time was found in the nasal and superior sectors of both rings. These findings are in agreement with those of Julia S. Steinberg et al, who observed that reticular pseudodrusen has predilection superior to the fovea[8], and with other previous studies reporting a reduction of ONL thickness over drusen[9-12].

The present study gives support to the finding of ONL thinning over time in areas with drusen, and the affectation seems to be higher in reticular pseudodrusen patients. Could these results explain the major functional impact of reticular pseudodrusen, compared with other types of drusen, even when good visual acuity exists? Querques et al evaluated the functional impact in microperimetry of reticular pseudodrusen, and found an impaired retinal sensitivity in this type of subjects compared with eyes with typical drusen. These authors concluded that this could be explained by a widespread disruption and loss of RPE and the inner-outer segments of photoreceptors interface associated with pseudodrusen deposits, rather than a focal outer retina impairment overlying drusen[15]. Furthermore, there are also different studies that document a strong relationship between reticular pseudodrusen and impairment in dark

adaptation in patients with early AMD[16-17]. The impact of reticular pseudodrusen on multifocal electoretinography (mfERG), an objective electrophysiological measure of retinal function, is not entirely clear. Although some studies reported a lack of relationship between mfERG and the presence of reticular pseudodrusen[18], other authors observed an association between functional changes and the presence of reticular pseudodrusen[19].

The results of the present study support the hypothesis that soft drusen and reticular pseudodrusen may produce photoreceptor thinning before the development of geographic atrophy or choroidal neovascularization, and may prove useful to further the understanding of the first symptoms of early stages of AMD, even before visual acuity is affected. Reticular pseudodrusen represent not only a risk factor for late AMD, but it is also a critical risk for retinal dysfunction.

The main limitation of this study was the relatively small number of eyes examined because of the strict inclusion and exclusion criteria, as well as the non-existence of a control group of healthy subjects to compare the results by using the same instrumentation and measurement methodology. Besides, another significant limitation was the difficulty in manual measurements in patients with high number of drusen. For future studies, it would be interesting to analyze the change of ONL in the same groups of patients, with a larger study sample and equally for both groups, with a control group to compare the significance of the changes, and to explore the relationship between anatomical changes and other aspects such as microperimetry, dark adaptation and ERG.

In summary, we found that both soft drusen and reticular pseudodrusen are associated with ONL thinning, with significant differences according to the type of drusen and sector in which they are located. Besides, this thinning is superior on the reticular pseudodrusen group. Overall, the main thickness changes, in both groups of patients, were found in areas where the prevalence of drusen is higher.

REFERENCES

(1) Armad, F., Universitario, H., Paz, L., Health, U., & View, E. (2011). Prevalence of age-related macular degeneration in Spain. *British Journal of Ophthalmology*, *95*(7), 931–936.

(2) Steinberg, J. S., Auge, J., Jaffe, G. J., Fleckenstein, M., Holz, F. G., & Schmitz-Valckenberg, S. (2013). Longitudinal analysis of reticular drusen associated with geographic atrophy in age-related macular degeneration. *Investigative Ophthalmology and Visual Science*, *54*(6), 4054–4060.

(3) Jager, R. D., Mieler, W. F., & Miller, J. W. (2008). Age-Related Macular Degeneration. *The New England Journal of Medicine*, *358*(24), 2606–2617.

(4) Sarks, J. P., Sarks, S. H., & Killingsworth, M. C. (1994). Evolution of soft drusen in age-related macular degeneration. *Eye*, *8*(3), 269–283.

(5) Mimoun, G.; Soubrane, G., & Coscas, G. (1990) Macular drusen. *Journal Français d'Ophtalmologie*, 13(10):511-530.

(6) Buitendijk, G. H. S., Hooghart, A. J., Brussee, C., de Jong, P. T. V. M., Hofman, A., Vingerling, J. R., & Klaver, C. C. W. (2016). Epidemiology of reticular pseudodrusen in age-related macular degeneration: The rotterdam study. *Investigative Ophthalmology and Visual Science*, *57*(13), 5593–5601.

(7) Saade, C., & Smith, R. T. (2014). Reticular macular lesions: a review of the phenotypic hallmarks and their clinical significance. *Clinical & experimental ophthalmology*, 42(9), 865-874.

(8) Steinberg, J. S., Fleckenstein, M., Holz, F. G., & Schmitz-Valckenberg, S. (2015). Foveal sparing of reticular drusen in eyes with early and intermediate age-related macular degeneration. *Investigative Ophthalmology and Visual Science*, *56*(8), 4267–4274.

(9) Sadigh, S., Cideciyan, A. V., Sumaroka, A., Huang, W. C., Luo, X., Swider, M., Jacobson, S. G. (2013). Abnormal thickening as well as thinning of the photoreceptor layer in intermediate age-related macular degeneration. *Investigative Ophthalmology and Visual Science*, *54*(3), 1603–1612.

(10) Early Treatment Diabetic Retinopathy Study Research Group. (1991). Grading diabetic retinopathy from stereoscopic color fundus photographs—an extension of the modified Airlie House classification: ETDRS report number 10. *Ophthalmology*, *98*(5), 786-806.

(11) Hanumunthadu, D., Wang, J. P., Chen, W., Wong, E. N., Chen, Y., Morgan, W. H., … Chen, F. K. (2016). Impact of retinal pigment epithelium pathology on spectral-domain optical coherence tomography-derived macular thickness and volume metrics and their intersession repeatability. *Clinical & Experimental Ophthalmology*, *45*(3), 270–279.

(12) Schuman, S. G., Koreishi, A. F., Farsiu, S., Jung, S. ho, Izatt, J. A., & Toth, C. A. (2009). Photoreceptor Layer Thinning over Drusen in Eyes with Age-Related Macular Degeneration Imaged In Vivo with Spectral-Domain Optical Coherence Tomography. *Ophthalmology*, *116*(3), 488–496.

(13) Curcio, C. A., Messinger, J. D., Sloan, K. R., McGwin, G., Medeiros, N. E., & Spaide, R. F. (2013). Subretinal Drusenoid Deposits in Non-Neovascular Age-Related Macular Degeneration. *Retina*, *33*(2), 265–276.

(14) Papay, J.A., Clark, C.A., Chui, T.Y., Zhao, L., & Elsner, A.E. (2012). Distribution of Outer Nuclear Layer Thickness in SDOCT Images. *Investigative Ophthalmology and Visual Science.* 53(14), 5664.

(15) Querques, G., Massamba, N., Srour, M., Boulanger, E., Georges, A., & Souied, E. H. (2014). Impact of Reticular Pseudodrusen on Macular Function. *Retina*, *34*(2), 321–329.

(16) Flamendorf, J., Agrón, E., Wong, W. T., Thompson, D., Wiley, H. E., Doss, E. L., & Cukras, C. (2015). Impairments in Dark Adaptation Are Associated with Age-Related Macular Degeneration Severity and Reticular Pseudodrusen. *Ophthalmology*, *122*(10), 2053–2062.

(17) Laíns, I., Miller, J. B., Park, D. H., Tsikata, E., Davoudi, S., Rahmani, S., & Husain, D. (2017). Structural Changes Associated with Delayed Dark Adaptation in Age-Related Macular Degeneration. *Ophthalmology*. doi: 10.1016/j.ophtha.2017.03.061

(18) Alten, F., Heiduschka, P., Clemens, C. R., & Eter, N. (2012). Multifocal electroretinography in eyes with reticular pseudodrusen. *Investigative Ophthalmology and Visual Science*, *53*(10), 6263–6270.

(19) Wu, Z., Ayton, L. N., Makeyeva, G., Guymer, R. H., & Luu, C. D. (2015). Impact of reticular pseudodrusen on microperimetry and multifocal electroretinography in intermediate age-related macular degeneration. *Investigative Ophthalmology and Visual Science*, *56*(3), 2100–2106.